IV. "On the Development of the Stigmata in Ascidians." By WALTER GARSTANG, M.A., Jesus College, Oxford; Berkeley Fellow of the Owens College, Manchester. Communicated by A. MILNES MARSHALL, M.A., M.D., F.R.S. Received April 14, 1892.

The respiratory organ or pharynx of the Tunicata exhibits a great amount of variability in form, size, and complexity of structure in the different members of the group—a variability which is obviously correlated with the physiological value of the organ. In the simplest forms, the Perennichordata or Appendicularians, the pharynx is a mere hollow cylinder, provided with a single pair of tubular gill-clefts, one on each side. In the higher forms (the Caducichordata) the cavities of the two gill-clefts become enormously dilated, so as to constitute a pair of peribranchial chambers interposed on either side between the pharynx and the body-walls. The dilatation of the gill-clefts to form peribranchial chambers can be aptly compared with the formation of branchial pouches in the tubular gill-clefts of Marsipobranch Fishes. But whereas the respiratory surface of the branchial pouches of Marsipobranchs is increased by folds of the walls of the pouches, the same purpose in the higher Tunicata is effected by a different means. The inner (visceral) walls of the peribranchial chambers apply themselves closely to the wall of the pharynx, and perforations then appear at numerous points, where the pharyngeal and peribranchial membranes have actually united. The remnants of the pseudo-cœlomic cavity, enclosed between the pharynx and the visceral walls of the peribranchial chambers, become extensive channels for a vigorous circulation of the hæmal fluid. The organ formed by the union of the pharyngeal and peribranchial walls is usually referred to as the "branchial sac;" and the perforations, which put the cavity of the pharynx into extensive communication with the peribranchial chambers, constitute the so-called "stigmata."

The stigmata vary much in form and arrangement. In the fixed Ascidians, whether simple or compound, they are usually simple slits, of a narrow elongated form, arranged in a series of rows placed transversely to the longitudinal axis of the body (*Ascidia*, *Clavelina*, *Botryllus*, *Styela*). In some genera, however (*Corella*, *Molgula*), the stigmata are curved and somewhat spirally arranged; but this condition is undoubtedly derived from the former by modifications of a secondary nature. In the pelagic Tunicata (*Salpa*, *Doliolum*, *Anchinia*, *Pyrosoma*), the condition met with in the fixed Ascidians is never found; there is never more than one row of stigmata on each side, and this row, though occasionally oblique or even transverse, is

usually longitudinal in direction. In *Pyrosoma* and some species of *Doliolum* the stigmata are narrow, elongated slits, extending transversely across the whole lateral face of the pharynx—each stigma occupying an area which in the fixed Ascidians is taken up by an entire transverse row of stigmata.

To ascertain what is the fundamental order underlying all these variations, and to determine what degree of correspondence and homology there is between the stigmata of the pelagic Tunicata and those of the fixed Ascidians, is not an easy matter; indeed, the possibility of any detailed comparison hardly seems to have occurred to the majority of investigators. Two views, due to Herdman and Lahille respectively, are, however, worthy of mention here.

Professor Herdman* derives *Pyrosoma* from a group of the compound Ascidians, through the curious colonial form *Cœlocormus Huxleyi*; and, in harmony with this view, he regards each of the transverse stigmata of *Pyrosoma* as corresponding to an entire transverse row of stigmata in the Ascidians, the several stigmata of the row having apparently coalesced to form the single stigma of *Pyrosoma* —a process which has almost certainly occurred in certain deep-sea types (*Fungulus*, *Culeolus*, &c.).

Lahille† characterises the latter portion of Herdman's view as a "profound error," and attempts, instead, to establish the remarkable proposition that the longitudinal row of transverse stigmata in *Pyrosoma* is strictly homologous with one of the transverse rows of longitudinal stigmata in an Ascidian, through a phylogenetic rotation of position. The oblique position of the row of stigmata in some species of *Doliolum* (*e.g.*, *D. Ehrenbergi*) is regarded as an intermediate condition between the two extremes. Lahille bases this proposition upon the changes of position which the organs of a *Pyrosoma*-bud undergo during development. These changes, it is true, are very remarkable, but they furnish absolutely no evidence for Lahille's contention; for it is a well-established fact‡ that immediately after their first appearance the stigmata of *Pyrosoma* begin to elongate in a direction at right angles to the long axis of the endostyle, and this relation is maintained through all the curious changes of form which the bud undergoes in its further development. Lahille's homologies are consequently without foundation; and, although Herdman's comparison is far more justifiable, yet the development of the transverse

* '"Challenger" Reports,' "Tunicata," 2nd Report, pp. 319, 320; 3rd Report, pp. 20, 24, 25, 137.

† 'Recherches sur les Tuniciers,' Toulouse, 1890, pp. 59, 61, figs. 44—51.

‡ Seeliger: "Zur Entwickelungsgeschichte der Pyrosomen;" 'Jenaische Zeitschrift,' vol. 23, 1889, p. 622, figs. 15, 17, 19. Salensky: "Beitr. z. Entwick. d. Pyrosomen;" 'Zoolog. Jahrbüch., Abth. f. Anat.,' vol. 5, 1891, p. 32. Salensky's figures 2 and 3, on p. 9, are, however, strangely inaccurate in this respect.

stigmata in *Pyrosoma* clearly tends to show that these are simple structures, and that they have not arisen by the modification of so many transverse rows of stigmata, as his theory demands.

The question is thus seen to be still unsolved, and if in this communication I venture to offer some observations upon the matter, it is in the belief that they tend considerably to elucidate the problem.

The view that the pelagic Caducichordate Tunicata have been profoundly modified in structure through their mode of life, and that they are to be derived phylogenetically from the so-called Compound Ascidians, is at present held, with varying reservations, by almost every recent investigator of the Tunicata, except Seeliger. It is held by Grobben and Uljanin for *Pyrosoma*, *Salpa*, and *Doliolum*, by Herdman for *Pyrosoma*, by Lahille for *Pyrosoma* and *Doliolum*, and by Salensky for *Pyrosoma* and *Salpa*. The evidence for this view has always seemed to me to be very slender and unimportant; and I believe it is this widely-spread conception which is answerable, among other things, for the absence of any satisfactory comparison between the stigmata of the fixed Ascidians and of their pelagic allies.

I have accordingly approached the question from the reverse point of view, believing that, by a study of the development of the stigmata in the fixed Ascidians, recapitulative stages would be met with which would furnish the desired answer. A grant awarded me by the Government Grant Committee last year enabled me, during the summer, to collect suitable material at the Plymouth station, and my observations have been completed in Professor Milnes Marshall's laboratories at the Owens College.

The development of the stigmata in the larva and oozooid of Ascidians has hitherto been very little investigated. The earliest complete account is that of Krohn,* an abstract of whose observations upon *Phallusia mammillata* is given by Balfour ('Comp. Emb.,' vol. 2, p. 20). Krohn's interpretations were subsequently criticised by E. van Beneden and Julin in their valuable paper† on the "Post-embryonic Development of *Phallusia* (*Ascidiella ?*) *scabroïdes*." These investigators showed that in the latter species two stigmata at first appear, one behind the other, on each side of the pharynx, and that subsequently new stigmata arise between and behind the two first, until a longitudinal row of six stigmata is formed on each side. These stigmata enlarge transversely to the long axis of the body, and subsequently subdivide, in the order of their formation, so as to constitute a corresponding number of transverse rows of smaller stigmata. Van Beneden and Julin thus drew a distinction between primary stigmata and secondary (definitive) stigmata, and called attention to the irregular order of formation of the primary stigmata as a point

* "Ueber die Entwicklung d. Ascidien," 'Müller's Archiv,' 1852.

† 'Arch. de Biologie,' vol. 5, 1884, p. 611.

worthy of notice. They did not, however, draw any general conclusions from the phenomena which they observed, beyond pointing out that the irregular order in which the primary stigmata appeared was in opposition to any theory as to their metameric arrangement.

The only other observations of importance are those of Seeliger* on the development of the stigmata in *Clavelina*. In this form, as in *Phallusia* and *Molgula*,† two pairs of stigmata at first arise, one behind the other, near the dorsal border of the sides of the pharynx. But instead of elongating in a transverse direction, as is the case in *Phallusia*, these stigmata elongate in a longitudinal direction, and become directly converted into the stigmata of the adult. With the downward extension of the peribranchial chambers, new stigmata arise independently, below the two first formed, so that eventually two transverse rows of perforations are formed on each side of the pharynx; and all these, by growth in a longitudinal direction, become directly converted into the slit-like stigmata of the adult. Subsequently, after the attachment of the larva, new transverse rows of stigmata arise in front of and behind the two first rows in an identical manner.

I have myself followed out the development of the stigmata in *Clavelina*, and have nothing to add to, or alter in, Seeliger's description; the stigmata invariably arise quite independently, and I have seen no indication of such a process of subdivision as has been described above for *Phallusia* (*Ascidiella ?*) *scabroïdes*.

A similar independent mode of origin of the stigmata has also been observed by Giard‡ in *Perophora*, and by Lahille§ in *Distaplia magnilarva*.

Thus, up to the present time, we are acquainted with three distinct genera in which the stigmata arise independently of one another; while the process of subdivision, described for *Phallusia* (*Ascidiella ?*) *scabroïdes*, remains unconfirmed and entirely without parallel. It would even be excusable to regard this latter method, from its exceptional character, as a developmental modification of the former. But before discussing this diversity of development, I will describe certain observations which I have made as to the development of the stigmata in several other types of Ascidians.

In *Botryllus* the stigmata of the adult have the usual form of

* "Zur Entwicklungsgesch. d. Socialen Ascidien," 'Jen. Zeit.,' vol. 18, 1885, pp. 45—150, Plates 1 to 8.

† P. J. van Beneden (*M. ampulloïdes*). Kupffer, 'Arch. f. Mikr. Anat.,' vol. 8, 1872, Taf. 17, fig. 8*a*. Lacaze-Duthiers, 'Arch. de Zool. Exp.,' vol. 3, 1874, pp. 623. 631, Plate 27.

‡ 'Arch. de Zool. Exp.,' vol. 1, 1872, p. 677, Plate 24, fig. 6.

§ 'Recherches sur les Tuniciers,' p. 165.

longitudinally elongated slits, arranged in a series of transverse rows on each side of the pharynx.

Various points in the development of *Botryllus* have been elucidated by the researches of Metschnikoff,* Krohn,† and Ganin,‡ but the development of the stigmata remains still undescribed.

The stigmata of the oozooid arise in a manner very different from that which we have seen in the case of the compound Ascidians *Clavelina*, *Perophora*, and *Distaplia;* the mode of their development recalls the phenomena described by E. van Beneden and Julin for "*Phallusia*" *scabroïdes*, but presents distinctive features of considerable importance.

In the earliest stage which has come under my observation, the young zooid (*B. aurolineatus*, Giard) is already fixed and is provided with the rudiments of two buds, one on each side. The endodermic vesicles of the buds as yet show no signs of differentiation. The zooid itself possesses the rudiments of two lateral tentacles only, and is provided with the eight club-shaped ectodermic processes, with long stalks, which are so characteristic of the larva.

The pharynx is provided with four pairs of transversely elongated stigmata, whose transverse diameters are nine times as great as their antero-posterior diameters. These huge transverse slits, whose width almost equals the length of the endostyle, extend right across the sides of the pharynx, from the dorsal region to the endostyle. They are not exactly of equal size, but decrease slightly in width in regular order from before backwards. The second slit is 0·2 mm. wide. The endostyle at this stage is 0·25 mm. long.

In the next stage examined (*B. aurolineatus*) the endodermic vesicle of each bud is already differentiated into a median pharyngeal portion and a pair of lateral peribranchial portions. The oozooid has now the rudiments of four tentacles, and the number of ectodermic processes has increased to eleven, the separate stalks being now very short.

The pharynx possesses, in place of the four pairs of transversely elongated stigmata, four transverse rows of small stigmata on each side. The anterior row is 0·42 mm. wide, the second row is 0·35 mm. wide, and the two posterior rows are still narrower. The endostyle at this stage is 0·425 mm. long. The first row consists of 10 stigmata, the second of 8 stigmata, the third of 6 or 7, the fourth of still fewer. None of the stigmata are elongated transversely; they are, for the most part, of an oval form, slightly elongated longitudinally, but towards the dorsal side they are more or less circular.

At a still more advanced stage (*sp. incert.*) the oozooid has attained

* 'Bull. Acad. Imp. Sci. St. Pétersbourg,' 1869, pp. 291—293.

† 'Arch. f. Naturgesch.,' vol. 35, 1869, pp. 190—196, 326—333.

‡ "Neue Thatsachen;" 'Zeit. f. Wiss. Zool.,' vol. 20, 1870.

very large dimensions, the number of ectodermic processes has increased to 36, and each of the lateral buds has given origin to a pair of buds of the second generation. At this stage there are five rows of stigmata on each side; but whether the fifth row is formed by the subdivision of a transverse primary stigma, or not, I have been unable to determine.

This is the most advanced stage in the progressive development of the oozooid of *Botryllus* that I have seen, and there is reason to believe that the number of rows of stigmata does not increase after this point. In a young colony, very little older than the one just described, the oozooid has undergone great reduction in size, and is evidently dying away, while the two buds of the first generation, to which it gave rise, have grown considerably in size, and are almost fully organised.

It is perfectly clear from the above account that in *Botryllus* the stigmata of the full-grown oozooid are secondary formations, due to the subdivision of a series of transversely elongated primary stigmata with which the larva is provided. It is also noteworthy that the primary stigmata—if an inference may be drawn from their relative sizes—arise one after another in regular order from before backwards, and that they are subsequently subdivided in the same order.

It will be convenient to distinguish the transversely elongated primary stigmata by some distinctive name, and on this account I propose for them the term "protostigmata." It cannot be denied that these structures present striking analogies with the true gill-clefts of *Amphioxus* and the lower Vertebrata.

Turning now to the buds of *Botryllus*, a remarkable difference is to be observed in the mode of origin of the stigmata, a difference which has important bearings upon the question of their phylogenetic history. Transverse protostigmata are never formed in the buds, whatever be the number of the generation to which the buds belong. The stigmata of the four first rows arise almost simultaneously as small rounded perforations which are entirely independent of one another; they soon begin to elongate in an antero-posterior direction and rapidly assume their definitive form.

Botryllus, therefore, exhibits both the modes of development which are known to occur in Ascidians; the stigmata in the oozooid arise by the subdivision of protostigmata, and the stigmata of the buds appear quite independently of one another. This fact renders it possible to determine which method is the more primitive. In any contrast of this sort between larval and bud development, there can be no doubt that it is the larva which exhibits the primitive mode, while the development in the bud is secondary and modified.

Now the development of the stigmata in the oozooids of *Clavelina* and *Distaplia* proceeds in essentially the same manner as in the buds

of *Botryllus;* and there can be, accordingly, just as little doubt that the development presented by the oozooids of these forms is also secondarily abbreviated. This conclusion is corroborated by the facts that in these genera and their allies the ova contain more food-yolk than is usual in Ascidians, and that the duration of the free-swimming larval stage is greatly reduced.

It is therefore obvious that the primitive (phylogenetic) mode of development of the stigmata in Ascidians is by the subdivision of transverse protostigmata arranged in a single longitudinal series on each side of the pharynx.

I have observed this process in two other species of Ascidians, *Thylacium sylvani* (Carus)* and *Styela* (*Styelopsis*) *grossularia* (van Beneden). These two species are very closely allied, and exhibit no appreciable differences in their mode of development.

In *Thylacium sylvani* eight protostigmata arise on each side of the pharynx, and become subdivided, in regular order from before backwards, to form a corresponding number of rows of secondary stigmata. The protostigmata extend right across the sides of the pharynx, as in *Botryllus*, before they are subdivided; and, although I have not actually observed their earliest stages, they give every appearance of having been formed in regular order from before backwards. The subdivision of the protostigmata begins towards their dorsal extremities and then extends ventrally—a process which compares very well with the formation of the stigmata in *Clavelina*. In the pharynx, at an early post-larval stage, and after the subdivision of the protostigmata has commenced, the actual nature of the process can easily be observed. Small projections arise from the anterior margins of the protostigmata, and are met by corresponding outgrowths from their posterior margins; the tips of these projections then coalesce with one another, and by their union give rise to the so-called interstigmatic bars of the fully constituted branchial sac. The shape of the secondary stigmata during the process of subdivision is often very irregular, but an admirable symmetry and regularity of form and arrangement is presented as soon as the subdivision is completed.

The following numbers illustrate the regular order in which the protostigmata are subdivided. They represent the numbers of secondary stigmata into which the protostigmata of one side have become converted in a pharynx 1·125 mm. long and 2·5 mm. in circumference, at a period when the stigmata of the first row have already assumed the form of narrow, longitudinally elongated slits:—

* I owe to Professor Ray Lankester the opportunity of examining the type-specimen of this species, which is in the collection of the Oxford University Museum. It was very erroneously described by its discoverer, and a redescription of it, which I have prepared, will be published shortly.

Row.	Number of stigmata.	Observations.
1	17	Length (antero-posterior) of stigmata, in middle of row, 0·25 mm.
2	16	Ditto, ditto, ditto, 0·125 mm.
3	10	Four are transversely elongated, one is round, and five are of a longitudinally oval form. Length, 0·075 mm.
4	3	One very wide stigma, and two longitudinally oval ones at the dorsal extremity.
5	2	One extremely wide stigma, and one small transversely elongated stigma at the dorsal extremity.
6 7 8	The three posterior protostigmata are completely undivided. The width of the 6th is 0·55 mm.; of the 7th, 0·425 mm.; and of the 8th 0·25 mm.	

The regular development of the protostigmata in *Botryllus* and *Thylacium* contrasts markedly with the phenomena observed in "*Phallusia*" *scabroïdes* by van Beneden and Julin, but it is very probable that the irregularity of formation in that species is the result of secondary changes. It may, I think, be safely concluded that the protostigmata of Ascidians arose primitively in regular order from before backwards.

It is very significant that in the pelagic Tunicate *Pyrosoma* the phylogenetic inferences which have here been drawn from the development of the stigmata in Ascidians are exactly fulfilled. In this form—as I have stated in the introduction—the stigmata are arranged in a single longitudinal series along each side of the pharynx, and they are transversely elongated, from the dorsal surface to the endostyle. They therefore resemble precisely, both in form and in arrangement, the protostigmata of larval Ascidians. Moreover, it appears from the recent researches of Salensky* that the stigmata in the ascidiozooids of *Pyrosoma* arise in regular order from before backwards, just as do the protostigmata in *Botryllus* and *Thylacium*. These resemblances are of too important a character to be mere coincidences. I would therefore submit that in *Pyrosoma* we have a primitive type of Caducichordate Tunicata, which is antecedent to the whole of the phylum Ascidiacea, and which exhibits very closely the ancestral form of pharynx from which the complicated respiratory organ of the fixed Ascidians has been derived.

It would further follow that *Clavelina* and its allies can no longer

* *Loc. cit.*, pp. 5, 33.

be regarded as the most primitive members of the order Ascidiacea, and that *Botryllus* and the Styelinæ must take this position; for in the structure and development of the pharynx, as well as in other points, with which I shall fully deal elsewhere, the latter forms approach, more nearly than any other Ascidians, the ancestral type represented by *Pyrosoma*.

V. "Observations on the Post-Embryonic Development of *Ciona intestinalis* and *Clavelina lepadiformis*." By ARTHUR WILLEY, B.Sc. Lond. Communicated by Professor RAY LANKESTER, M.A., F.R.S. Received May 4, 1892.

The following is an account of some of the observations which were made by the author during an occupation of the British Association Table at the Zoological Station at Naples from October, 1891, to May, 1892.

In their admirable "Recherches sur la Morphologie des Tuniciers," ('Archives de Biologie,' vol. 6, 1887), Édouard van Beneden and Charles Julin came to a number of conclusions which, while they appeared to follow naturally from the facts observed, yet only added, if possible, to the perplexity surrounding any attempt to regard the Ascidians and *Amphioxus* from a common standpoint. Led away by the remarkable behaviour of the endostyle which I observed and described in the larva of *Amphioxus*, I easily induced myself to accept the views of the Belgian *savants*.

The observations on the post-embryonic development of *Ciona* described below oblige me, however, to reconsider the position which I took in my paper on "The Later Larval Development of *Amphioxus*" ('Quart. Journ. Micro. Sci.,' vol. 32, 1891), with regard to the mutual relations of the Ascidians and *Amphioxus*, and may, I hope, tend to the establishment of reasonable homologies between them.

It is necessary to recapitulate very briefly the views of van Beneden and Julin, in order to bring those which I am about to oppose to them in the most striking contrast.

The following table shows at a glance the homologies suggested by the above-named authors:—

(*a.*) The anterior intestinal diverticula of *Amphioxus*, the right one of which becomes the large head- or, better, proboscis-cavity, while the left becomes the præoral pit. = The primary branchial canals of Ascidians (*i.e.*, the first pair of gill-slits; see below).